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Robustness of the signal transduction system of the mammalian JAK/STAT pathway and dimerization steps.

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1. Focus Response - The Story.

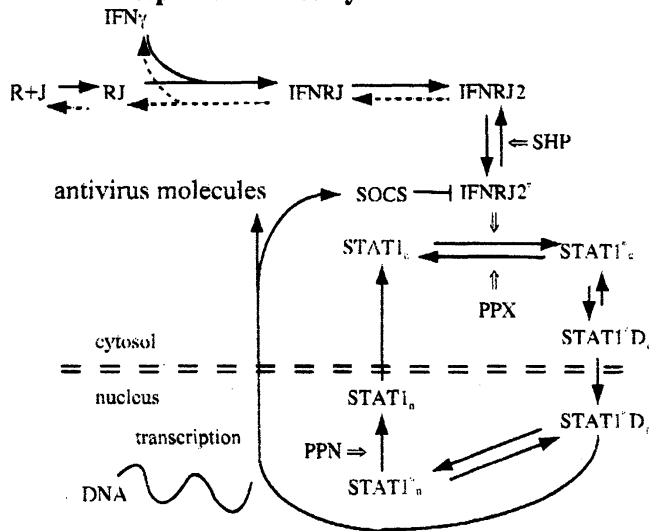


Fig. 1 Diagram for JAK/STAT1 pathway.

The JAK/STAT pathway is stimulated by interferons (IFNs) and eventually induces the expressions of a number of genes that inhibit virus replication and increase expression of MHC molecules. In Fig. 1A, prior to ligand binding, neither receptor nor STAT1 is phosphorylated. All the STAT1 molecules are

monomers and are in the cytosol. While ligands are bound to their receptors, STAT1 molecules are recruited from the cytosol and then subsequently phosphorylated. Phosphorylated STAT1s form homodimers and hetero-dimers with unphosphorylated STAT1, which move to the nucleus. They then bind to particular sequences and activate multiple genes, coding for SOCS, which is a negative regulator of the JAK/STAT pathway, as well as proteins that lead to the enhancement of defense. Details are shown in Shudo *et al.* (in review).

2. The Optimal Control.

What is the role of this requirement of dimerization? Potential answers could be given from the perspective of the "optimal control". IFN γ activate and recruit more mononuclear phagocytic cells to the site of infection, resulting in the formation of granulomas¹. The input signal, IFN γ , might include random fluctuation caused by the noises in living cells, partly because the smallness of the number of molecules per cell for genes, RNAs and proteins causes stochasticity in chemical reactions². If the receptor reacts to the noise (small size of the input) with too much sensitivity, the downstream reactions are started in the absence of a real microbial infection. Such a false alarm could lead to the abnormal formation of granulomas and cause considerable cost and harm to the host. On the other hand,

if a signal (large size of the input) arrives, the system needs to react properly to suppress the microbial growth as soon as possible. Ideally, the system is expected to react only to clear signals of interferons, but avoid reacting to small and irregular noises. In addition, JAK/STAT1 pathway must be able to respond to the large size of the input with a short time delay, as the pathogen is expected to proliferate. Otherwise, huge amounts of antiviral molecules will be needed to kill the pathogen at the site of infection. Also, the reaction must be relatively insensitive to the noises inherent in its own reaction. It is because non-lethal substitutions of amino acids can happen frequently. That could change the strength of the affinity between proteins and between genes and proteins.

Taken together, we explore system properties such as switch-like reaction, delay, and parameter sensitivity; to clarify the role of the complex structures observed in JAK/STAT1 pathways of mice. We compare the original model based on experiments and observations³, with several alternative models in which some dimerization steps are not required to activate genes, coding antiviral molecules and SOCS.

3. Model

3.1 Difference between the original and alternative models.

In the model in Yamada *et al.* (2003)³ the phosphorylated STAT1 must be homodimerized before it can either enter the nucleus or activate the transcription of genes. To examine the effect of the dimerization steps, we compare the original model and those with some of these steps altered. To determine the relative importance of the two-dimerization steps of phosphorylated STAT1, one occurring just before translocation to the nucleus and the other just before gene expression, we study models, on which one or both of these steps are not required for gene activation, and compare them with the original model. Therefore, there are four models as shown in Table 1.

	The form of STAT induces the expression of genes coding antiviral molecules.	The form of STAT translocates to the nucleus.
The original model (Yamada et al.)	dimers	dimers
The alternative model 1	dimers	monomers
The alternative model 2	monomers	dimers
The alternative model 3	monomers	monomers

Table 1. Original model based on experiments and modified models.

3.2. Parameter estimate for 4 models.

Estimated values of parameters by Yamada *et al.* (2003)³ were based on the structure of the original model. In contrast, four models have different structure from each other. Accordingly, we have to re-estimate values of parameters for each model. For the estimate,

we used the Levenberg-Marquardt algorithm, with the digitized data of STAT1_c, i.e. unphosphorylated STAT1 monomers in the cytosol, and STAT1^{*}D_n, i.e. homodimers of phosphorylated STAT1 in the nucleus, shown in Fig. 2 of Yamada *et al.* (2003)³. In this paper, the asterisk shows that the molecule is in a phosphorylated form. "D" indicates that this is a homodimer.

4. Results and Discussions.

If the system observed in the organism is designed to achieve the most robust system, the original model should be more robust than any alternatives. We categorized the property of different models into 2 groups -- "+" or "-", as shown in Table 3.

4.1 Signal-Response curve and the Hill Constant

We obtained the concentration of antiviral molecules at the steady state, for different numbers of initial IFN γ , or signal response curve. The Hill constant values are: $n = 3.98$ for the original model; $n = 3.89$ for the alternative model 1; $n = 3.75$ for the alternative model 2; and $n = 1.94$ for the alternative model 3. The alternative model 3 which had the smallest Hill constant was categorized into "-". The other models which had similar Hill constant to each other, were "+".

4.2 Delay

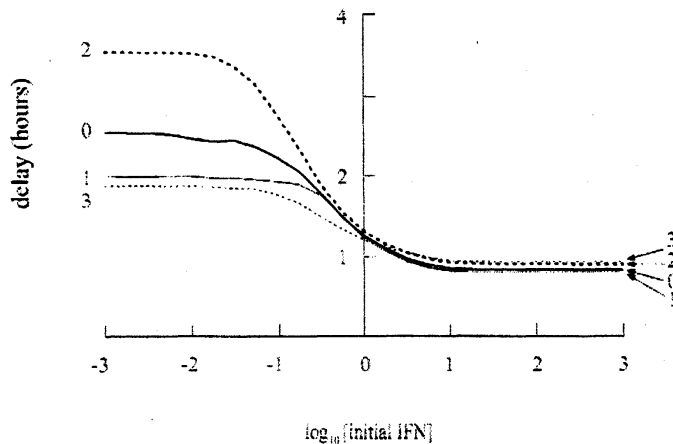


Fig. 2 Time delay in the production of antiviral molecules, L_i . Dimerization requirement increases the delay in the region of small size of the input, while it decreases in the region of large size of the input.

We focused on the delay L_i , or time point at which the concentration of antiviral molecules first reaches 50% of the concentration at the steady state, against the small size of the input (noise) and signal, or large size of the input (Fig. 2). In the region of the small size of input, $L_2 > L_0 > L_1 > L_3$ held. The alternative model 1 and 3 which had the shortest or the second shortest delay L_i upon the small size of the input, were categorized into "-". The other models, which had the longest or second longest delay L_i were "+". In the region of the large size of input, $L_3 \approx L_2 > L_0 \approx L_1$ was held. The alternative model 2 and 3 which had long

delay L_i , upon the large size of the input, were categorized into "-". The other models, which had short delay L_i , were "+". It is noted that the difference of delay between models is smaller than that in the region of a low numbers of $IFN\gamma$.

4.3 The parameter sensitivity

We compared the parameter sensitivity with respect to noise inherent in its own reaction. The parameter sensitivity is defined as

$$S_{par} = \frac{\partial \log_e(a.v.)}{\partial \log_e(par)} \bigg|_{\substack{a.v.=\overline{a.v.} \\ par=par}} \quad (1)$$

where \overline{par} are parameter values, which are estimated for each model in this paper, and $\overline{a.v.}$ is the concentration of antiviral molecules at the steady state given by those sets of parameters. The Δpar and $\Delta a.v.$ are deviations from those standard values.

No	description	original	model 1	model 2	model 3
Dephosphorylation of STAT1 in nucleus					
1	Total of PPN	-0.013	-2.025	-1.017	-1.035
2	Association of PPN with STAT1*n or STAT1*Dn	-0.013	-2.025	-1.017	-1.035
3	Reverse reaction of [2].	0.012	1.950	0.984	0.999
4	Dephosphorylation of STAT1*n or STAT1*Dn by PPN	-0.012	-1.985	-0.989	-1.009
Dimerization of STAT1 in nucleus and in cytosol.					
13	Homo-dimerization of STAT1*c (STAT1*n)	0.982	0.987	1.011	0.000
14	Reverse reaction of [13].	-1.957	-0.983	-0.570	0.000
15	Hetero-dimerization of STAT1c (STAT1n) and STAT1*c (STAT1c)	0.000	0.000	-0.106	0.000
16	Reverse reaction of [15].	0.000	0.000	0.110	0.000

Table 2. Sensitivity with respect to each parameter. PPN; nuclear phosphatase of STAT1.

We calculated the magnitude of sensitivities to all 43 parameters included in reactions of the JAK/STAT1 pathway, under the condition that the $IFN\gamma$ number is low ($=10^{-3}$ nM).

The sensitivity with respect to parameters associated with hetero-dimerization of STAT1* (number 15 and 16) was much larger (100 times or more) in the alternative model 2 than any other models (original model, and alternative models 1, 3), whilst the other models

did not have such a disadvantage.

On the other hand, the sensitivity with respect to parameters associated with nuclear phosphatase of STAT1 (number 1-4) was much smaller (1/100 times or less) in the original model than in the other models (alternative model 1, 2, and 3). In addition, the alternative model 3 has such an advantage, and it is robust to the perturbations of parameters associated with homodimerization of STAT1* (number 13-14). It is noted that the alternative model 1 and 2 did not have such an advantage. In conclusion, the original model and the alternative model 3 are more robust than the others to the perturbations of parameters included in reactions of the JAK/STAT1 pathway. Especially, the alternative model 2 is unstable to the perturbations of parameters.

4.4 Summary of results

Following are summaries of properties of each model. The original model was categorized into "+" in all aspects, while any alternative models were not. In conclusion, only the original model could be robust with respect to all important aspects, switch-like response, control of delay depending on the size of the input, and low sensitivity to the noise inherent in its own reactions.

	original	model 1	model 2	model 3
Switch-like response	+	+	+	-
Delay (noise)	+	-	+	-
Delay (signal)	+	+	-	-
Robustness against noise inherent in its own reaction. (noise)	+	+/-	-	+

Table 3. Property of each model.

5. Researches related to this paper.

In the present paper, we assume that the robust system needs to the switch-like response to signal, instead of the responses that amplify the noise^{4,5}. It is based on the idea that switch-like response is a profitable manner in which the defense system responds to pathological stress. For example, Jun N-terminal kinase (JNKs) which induces apoptosis in different organisms, shows steep switch-like response to hyperosmolar sorbitol (Hill constant is 20)⁶. A similar idea was proposed previously and was named as "ultrasensitivity"⁶⁻¹⁰. "Ultrasensitivity" is generated by a multistep of reactions^{1, 7-10}, saturation effect of the enzyme (zero-order)¹¹, and positive feedback¹². Most studies on ultrasensitivity have focused on linear cascades (MAPK and JNK cascade) or simple cyclic systems¹³⁻¹⁵. In contrast, JAK/STAT pathway, which we focus on in this paper, has many branches and a sophisticated loop of negative feedback by SOCS. In addition, "decisiveness" which has been discussed in the S-system, is also the same idea¹⁶.

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